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Pulmonary capillary blood volume and membrane conductance in Andeans and lowlanders at high altitude: A cross-sectional study

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ABSTRACT

Lung carbon monoxide (CO) transfer and pulmonary capillary blood volume (Vc) at high altitudes have been reported as being higher in native highlanders compared to acclimatised lowlanders but large discrepancies appears between the studies. This finding raises the question of whether hypoxia induces pulmonary angiogenesis.

Eighteen highlanders living in Bolivia and 16 European lowlander volunteers were studied. The latter were studied both at sea level and after acclimatisation to high altitude. Membrane conductance (Dm_{CO}) and Vc, corrected for the haemoglobin concentration (Vc_{cor}), were calculated using the NO/CO transfer technique. Pulmonary arterial pressure and left atrial pressures were estimated using echocardiography.

Highlanders exhibited significantly higher NO and CO transfer than acclimatised lowlanders, with Vc_{cor}/VA and Dm_{CO}/VA being 49 and 17% greater (VA: alveolar volume) in highlanders, respectively. In acclimatised lowlanders, Dm_{CO} and Dm_{CO}/VA values were lower at high altitudes than at sea level. Echocardiographic estimates of cardiac output and pulmonary arterial pressure were significantly elevated at high altitudes as compared to sea level.

The decrease in Dm_{CO} in lowlanders might be due to altered gas transport in the airways due to the low density of air at high altitudes. The disproportionate increase in Vc in Andeans compared to the change in Dm_{CO} suggests that the recruitment of capillaries is associated with a thickening of the blood capillary sheet. Since there was no correlation between the increase in Vc and the slight alterations in haemodynamics, this data suggests that chronic hypoxia might stimulate pulmonary angiogenesis in Andeans who live at high altitudes.

Introduction

Chronic hypobaric hypoxia is a cause of pulmonary hypertension in Andeans, which develops through the combined effects of hypoxic vasoconstriction, arteriolar remodelling, and polycythemia [1]. It has long been thought that pulmonary vascular rarefaction may be an additional contributing factor [2,3], but this notion has now been revised after a recent study concluded that rats placed in a hypoxic environment exhibit increased capillary diameter and length [4]. Thus, exposure to high altitude may be associated with improved gas exchange because of hypoxia-induced capillary angiogenesis.

In humans, the pertinent literature suggests major discrepancies in CO diffusion data that can be ascribed to both methodology, including differences in the techniques used to measure diffusion, and biology, such as differences in ethnicities or durations of exposure to high altitudes in lowlanders [5–12]. Most studies on diffusing capacity at high altitudes were performed more than 30 years ago. The two transfer components (Dm_{CO} and Vc) were calculated in these studies using measured TL_{CO} data along with two inspired oxygen fractions. These changes in oxygenation may potentially alter ventilation and pulmonary hypoxic vasoconstriction. The more recent one-step NO/CO method takes advantage of large differences in the diffusion properties of two diffusible gases. These large differences allow for easy computation of the membrane and capillary components of lung diffusion [13] while the oxygen

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fraction does not change appreciably. In our study, this method was applied to long-term high altitude residents and to subjects living at sea level who had recently moved to a high altitude. We tested the hypothesis that chronic hypoxic exposure in native highlanders would induce an increase in the capillary component of lung diffusion without haemodynamic changes.

Experimental procedures

Study design

A cross-sectional study was conducted on the Andean Plateau (Bolivia) which compared 18 subjects natives of the high Andean Plateau with 16 healthy European lowlanders acclimated to high altitude.

Subjects

Highlanders (14 males, 4 females), all natives of the high Andean plateau and living in the city of Oruro (Bolivia) or nearby, agreed to participate in this study. The altitude in this region is approximately 4000 m above sea level. The protocol was approved by the ethical committee of the Oruro Hospital and all subjects gave their informed consent. None of them were smokers. Ten of the 18 subjects complained of fatigue, dyspnea, headache, dizziness, and poor quality of sleep, all of which are symptoms compatible with a diagnosis of chronic mountain sickness, or "Monge disease" [1]. Patients with a history of thrombosis were excluded from the protocol. The eight remaining subjects (asymptomatic group) did not complain of any symptoms, although some were polycythemic. Vc and other related data derived from the measurement of CO and NO transfers were compared to those of the 16 healthy European lowlanders (8 males, 8 females) who had been acclimated to high altitude for four days at 4000 m. For the latter group, transfer values obtained at high altitude were also compared with sea level values. In lowlanders, measurements at high altitude were made in La Paz (3750 m).

Materials and methods

Transfer measurements

NO and CO transfer were measured using the single breath method, in the sitting position, as described elsewhere [13]. Reproducibility and repeatability of TL_{NO}, TL_{CO}, Dm_{CO} and Vc using this method has been established [14,15]. An automated apparatus performing calibrations, extemporaneous mixture of the gases and online calculations was used (Hypercompact, Medisoft, Dinant, Belgium). Measurements were repeated two to three times with the aim of obtaining TL_{CO} values within 5% and TL_{NO} values within 10% of each other. Two highlanders who had non-reproducible measurements were excluded from our study.

Diffusion capacities for NO and CO were obtained simultaneously from the exponential disappearance rate of each gas with respect to helium using the Jones and Meade method [16]. Dm_{CO} and Vc were calculated from the model and equation of Roughton and Forster [17]. This model permitted the transfer of carbon monoxide through the alveolo-capillary structure to be split into two resistances, one for the alveolar membrane (Dm_{CO}) the other for the blood reacting with the gas ($1/\theta_{CO} \times Vc$) as shown in then following equation: $1/TL_{CO} = (1/Dm_{CO}) + 1/\theta_{CO} \times Vc)$.

To solve the Roughton–Forster equation, the equation of Forster [18] for the conductance of CO with haemoglobin (θ_{CO}) was chosen ($1/\theta_{CO} = 1.3 + 0.0041 \times PcapO_2$) where $PcapO_2$ is the mean capillary oxygen pressure. $PcapO_2$ was estimated using $PAO_2 -$ (oxygen consumption/($TL_{CO} \times 1.23$)). The barometric pressure and the fraction of O_2 in the expired sample were taken into account to calculate PAO_2. Oxygen consumption was calculated by taking the mass balance of oxygen between inspiration and expiration during the manoeuvre. The fraction of oxygen in the residual volume preceding inspiration was assumed to be similar to that found in the expired sample (mean 16.4%). $TL_{CO} \times 1.23$ was chosen to substitute for TL_{O2}.

As some of the highlanders included in the study complained of symptoms compatible with Monge disease, an arterial blood sample was required to detect arterial hypoxemia or hypercapnia as a venous blood sample was sufficient to measure the haemoglobin in lowlanders. Arterial blood samples were withdrawn from the radial artery. Arterial blood gases and haemoglobin concentrations [Hb] were measured (Roche OPTI CCA, Holliston, USA). Lowlanders' haemoglobin concentrations were measured at high altitude and at sea level. Since the derived calculated value of Vc depends on haemoglobin concentration, all Vc figures were corrected (Vc_{cor}) for a reference value of haemoglobin concentration [Hb ref] of 14.6 g/dl for men and 13.4 g/dl for women.

Echocardiography

An echocardiographic examination was performed in all subjects using a portable vivid ultrasound system (GE Ultrasound, Norway). Lowlanders were studied at sea level the week before departure and at high altitude in La Paz. Highlanders were studied in Oruro. Cardiac output (Q') was calculated from the velocity–time integral recorded by pulsed Doppler in the left ventricular (LV) outflow tract. Mean pulmonary artery pressure (Ppa) was estimated from the acceleration time of the Doppler flow profile in the pulmonary artery [19]. Estimated pulmonary artery occluded pressure (Ppao) was calculated from the ratio between mitral inflow early diastolic wave maximal velocity (E) and the mitral annulus early diastolic wave maximal velocity (Ea) [20,21].

Data analysis

Results are expressed as mean ± SD data were analysed using paired and unpaired Student t-tests. Correlations were calculated using univariate linear regression analysis. $P < 0.05$ was considered significant.

Results

Subject characteristics

Table 1 lists anthropometric characteristics, blood gas data, and haemoglobin concentrations for our test subjects. The mean

Table 1
Characteristics of the subjects

	Highlanders			Lowlanders
	Symptomatic	Asymptomatic	All subjects	
Age (years)	49 ± 11	39 ± 12	45 ± 12	36 ± 13
Height (cm)	162 ± 10	167 ± 5	165 ± 8	170 ± 8*
Weight (kg)	74 ± 14	69 ± 15	72 ± 14	64 ± 12
BMI (kg m^2)	28.4 ± 6.1	24.7 ± 4.6	26.2 ± 5.7	21.9 ± 2.4*
PH	7.43 ± 0.05	7.46 ± 0.03	7.46 ± 0.04	–
$PaCO_2$ (mmHg)	36 ± 11	29 ± 4	33 ± 9	–
PaO_2 (mmHg)	45 ± 10§	53 ± 5	49 ± 9	–
Hb	18.1 ± 2.7	17.1 ± 1.7	17.7 ± 2.3	14.1 ± 1*
Hct (%)	54.7 ± 7.3	51.4 ± 5.1	53.6 ± 6.4	–

Values are means ± standard deviation. BMI, body mass index; PaO_2 and $PaCO_2$, partial pressures of arterial oxygen and carbon dioxide; Hb, haemoglobin concentration; Hct, hematocrit.
§ Significantly different from asymptomatic group.
* Significantly different from all highlander subjects.

haemoglobin concentration of the highlanders was higher than that of the lowlanders.

The only difference between the two groups of highlanders was the arterial PaO_2, which was lower in the symptomatic group ($P = 0.01$). Some patients in the symptomatic group exhibited relatively low Hb concentrations since they had recently undergone phlebotomy. Conversely, some subjects in the non-symptomatic group exhibited Hb concentrations that were close to those of the symptomatic group.

NO/CO transfer and echocardiographic variables in acclimatised lowlanders

The mean barometric pressure was 495 mmHg. The mean alveolar fraction of oxygen in the expired sample was 16.4%, i.e., PAO_2 was 73 mmHg. Pulmonary transfer variables at sea level and after four days of adaptation to high altitude are listed in Table 2. Variables are expressed as raw data and as percentages of sea level reference values (%SL) [22]. Dm_{CO} and Vc are also expressed per unit of alveolar volume (VA).

Lowlanders had an alveolar volume that did not vary from predicted values [23] and did not change with higher altitudes. However, mean TL_{NO}, Dm_{CO}, TL_{NO}/TL_{COcor}, Dm_{CO} /VA, and Dm_{CO} /Vc_{cor} decreased while neither TL_{COcor} nor Vc_{cor} changed.

High altitude exposure in lowlanders was associated with an increase in heart rate, LV ejection fraction, cardiac output, and Ppa without any change in estimated Ppao (Table 3).

NO/CO transfer and echocardiographic variables in highlanders

Pulmonary transfer variables in highlanders are listed in Table 4. No difference was found between symptomatic and asymptomatic groups.

Five of the 18 highlanders exhibited higher VAs than predicted values at sea level (SL) (>120% SL). One overweight highlander exhibited a lower than normal lung volume (73% SL). Mean Vc, expressed as a percentage of reference values, was 67% greater in highlanders than in acclimatised lowlanders since the difference in Dm_{CO} was only 25%. The ratio of Dm_{CO} to Vc_{cor} was also, on average, below the lower limit of the reference SL value (0.64) [22] and the mean was lower than in the lowlander group (0.52 ± 0.12 vs. 0.59 ± 0.06; $P = 0.03$). The relationship between Dm_{CO} and Vc_{cor} was linear in both populations (Fig. 1). The slopes of the regression lines were not different even though the data from the highlander groups extended to higher Vc values. The mean Vc_{cor}/VA was increased by 49% in highlanders as compared to lowlanders. Sixteen out of 18 had a Vc_{cor}/VA ratio above the upper limit of the reference values [22]. Dm_{CO}/VA in the highlanders was also significantly greater (+17%) than in lowlanders.

Table 3
Echocardiographic measurements in lowlanders at sea level and high altitude and in highlanders.

	Lowlanders Sea level	Lowlanders High altitude	Highlanders
HR (bpm)	63 ± 12	76 ± 13*	75 ± 12
Q' (L min^{-1} m^{-2})	2.8 ± 0.2	3.3 ± 0.7*	3.2 ± 0.7
Ppa (mmHg)	10.5 ± 4.2	29.6 ± 4.7*	28.7 ± 8.0
LV ejection fraction (%)	69 ± 8	77 ± 7*	67 ± 8§
Estimated Ppao (mmHg)	8.6 ± 1.6	7.9 ± 1.6	10.2 ± 3.3§

Values are mean ± standard deviation. Q', cardiac output; Ppa, mean pulmonary artery pressure; LV, left ventricular; Ppao, estimated pulmonary artery occluded pressure.
* Lowlanders at high altitude significantly different from at sea level.
§ Significantly different from lowlanders at high altitude.

Highlanders had similar heart rates, Q's and Ppas as acclimatised lowlanders, but the estimated Ppao was slightly higher among highlanders, though still well within the normal range. The correlation between Vc_{cor} and Ppao was negative and not significant ($P = 0.10$).

Discussion

The main finding of this study is that Vc_{cor} and Vc_{cor}/VA are elevated in people living at 4000 m above sea level, suggesting that chronic hypoxia induces distension of the capillary bed and/or recruitment of capillaries. This increase in Vc is observed in non-symptomatic highlanders as well as in those with Monge disease. Another important finding is the decrease in TL_{NO} (or Dm_{CO}) in lowlanders at high altitudes, expressed either as an absolute value or in relation to alveolar lung volume.

Methodological aspects

In the Roughton–Forster model [17], the calculations of Vc and Dm_{CO} introduce an equation for θ_{co}, i.e., the conductance of blood for CO. The relationship between $1/\theta_{co}$ and the partial pressure of O_2 is linear and positive in the 0–600 mmHg range. Several linear relationships have been identified in the past [18,24,25]. The choice of the in vitro Forster θ_{co} equation for this analysis has been discussed elsewhere [13,22].

$PcapO_2$ can either be set at an approximate value or estimated using the equation cited in the methods section [$PAO_2 - (\text{oxygen consumption}/(TL_{CO} \times 1.23)$]. The calculation remains an estimate

Table 2
Comparison of lung diffusion variables at sea level and at high altitude in the lowlander group.

Variable	Sea level		High altitude		
	Value	% SL pred.	Value	% SL pred.	*P*-value
$PcapO_2$ (mmHg)	118 ± 14	–	65 ± 8	–	0.000
TL_{COcor} (mL min^{-1} mmHg^{-1})	39 ± 7	116 ± 14	38 ± 9	112 ± 19	NS
TL_{NO} (mL min^{-1} mmHg^{-1})	170 ± 36	106 ± 12	143 ± 34	89 ± 14	0.000
Dm_{CO} (mL min^{-1} mmHg^{-1})	86 ± 18	106 ± 12	72 ± 17	89 ± 14	0.000
Vc_{cor} (ml)	125 ± 20	126 ± 20	125 ± 35	124 ± 27	NS
VA (L)	6.3 ± 1.1	105 ± 7	6.17 ± 1.2	104 ± 11	NS
TL_{NO}/TL_{COcor}	4.44 ± 0.3	–	3.8 ± 0.2	–	0.000
Dm_{CO}/VA	13.8 ± 2.3	105 ± 12	11.8 ± 1.6	90 ± 9	0.000
Vc_{cor}/VA	20.4 ± 4.6	125 ± 21	20.1 ± 3.5	124 ± 18	NS
Dm_{CO}/Vc_{cor}	0.69 ± 0.1	–	0.59 ± 0.06		0.002

Values are means ± standard deviation. Percent SL pred., percentage of predicted value at sea level; TL_{COcor}, transfer lung capacity for carbon monoxide corrected for haemoglobin concentration; TL_{NO}, transfer lung capacity for nitric oxide; Vc_{cor}, pulmonary capillary blood volume corrected for haemoglobin concentration; Dm_{CO}, diffusing capacity of the lung; VA, alveolar volume; *P*-value, comparison between sea level and high altitude raw data.

Table 4
Lung diffusion variables in the highlander group.

Variable	Symptomatic		Asymptomatic		All		
	Value	% SL pred.	Value	% SL pred.	Value	% SL pred.	*P*-value
$PcapO_2$ (mmHg)	66 ± 12	–	61 ± 10	–	63 ± 11	–	NS
TL_{COcor} (mL.min^{-1} mmHg^{-1})	49 ± 15	*163 ± 36*	52 ± 14	153 ± 34	50 ± 14	159 ± 35	0.004
TL_{NO} (mL.min^{-1} mmHg^{-1})	175 ± 62	*122 ± 32*	187 ± 41	114 ± 20	180 ± 53	119 ± 75	0.02
Dm_{CO} (mL.min^{-1} mmHg^{-1})	89 ± 31	*122 ± 32*	95 ± 21	114 ± 20	92 ± 27	119 ± 75	0.02
Vc_{cor} (ml)	173 ± 47	*196 ± 45*	189 ± 63	185 ± 57	180 ± 54	191 ± 49	0.001
VA (L)	6.6 ± 1.6	*113 ± 26*	6.7 ± 0.8	106 ± 10	6.6 ± 1.3	110 ± 20	NS
TL_{NO}/TL_{COcor}	3.56 ± 0.31	–	3.61 ± 0.39	–	3.6 ± 0.3	–	0.038
Dm_{CO}/VA	13.3 ± 2	*107 ± 15*	14.1 ± 2.2	111 ± 15	13.7 ± 2.1	108 ± 14	0.005
Vc cor/VA	26.6 ± 4.5	*172 ± 27*	28.2 ± 8.5	176 ± 53	27.3 ± 6.4	174 ± 39	0.000
Dm_{CO}/Vc_{cor}	0.51 ± 0.09	–	0.53 ± 0.14	–	0.52 ± 0.12	–	0.003

Values are mean ± standard deviation. Percent SL pred., percentage of predicted value at sea level; TL_{COcor}, transfer lung capacity for carbon monoxide corrected for haemoglobin concentration; TL_{NO}, transfer lung capacity for nitric oxide; Vc_{cor}, pulmonary capillary blood volume corrected for haemoglobin concentration; Dm_{CO}, diffusing capacity of the lung; VA, alveolar volume; *P*-value, comparison between highlander and lowlander altitude raw data.

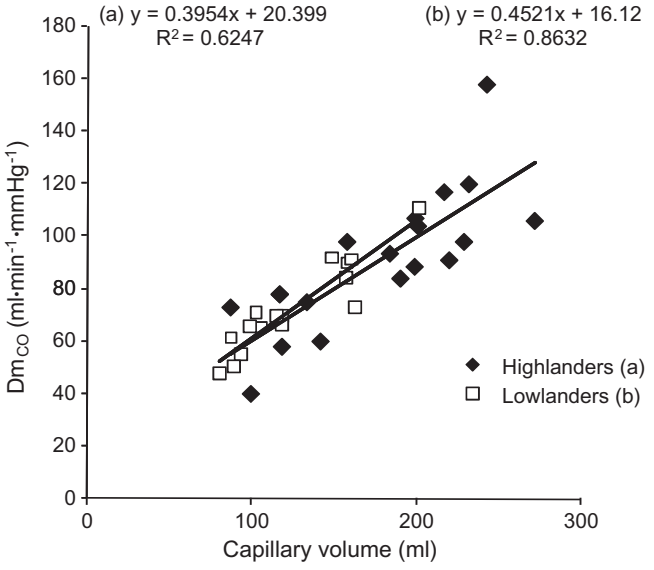

Fig. 1. Diffusion capacity of the lung as a function of pulmonary capillary blood volume in highlander (4000 m) and lowlander subjects. The slopes of the two regression lines were not different ($P > 0.5$).

since the mean PAO_2 during the single breath manoeuvre is not known. The expired sampled PO_2, used as a surrogate for PAO_2, slightly underestimates the true mean value of alveolar PO_2 during the short period of apnoea. The coefficient relating TL_{CO} to TL_{O2} is also a matter of debate. The generally used value of 1.23 takes into account differences in the physical properties of the two gases, but not the difference in blood conductance, [18,26–28]. Finally, since oxygen consumption during the test was low, the error in TL_{O2} estimates was a few mmHg.

It is suggested that the haemoglobin structure of Andeans who settled in high altitude regions at least 10,000 years ago may be altered and that their blood conductance for CO is therefore higher than normal. However, there is no experimental evidence for such a change in structure that would likely be associated with a change in the affinity of haemoglobin for oxygen. The affinity of haemoglobin for oxygen in Andeans, as estimated by the measurement of P50, has not been shown to be different from that of lowlanders [29]. In an additional study CO reactivity with haemoglobin has been checked on samples of haemoglobin of Bolivians raised at altitude but living in Europe. No difference has been found compared with European haemoglobins (see Annex).

Effect of hypoxia on membrane diffusing capacity

The decrease in TL_{NO} observed in lowlanders at high altitude can be interpreted either as an increase in the path length for the diffusion of gases in the gas phase or as a decrease in membrane conductance.

The latter hypothesis, which suggests possible interstitial oedema, seems less likely for several reasons. First, measurements were made on subjects at rest who had been only moderately active during the day. Second, none of the subjects experienced pulmonary symptoms that are compatible with pulmonary oedema, either during their stay at high altitudes or subsequently during exercise. Finally, the fact that all subjects exhibited approximately the same percentage of decrease in TL_{NO} is in disagreement with the hypothesis of scattered incidence of interstitial oedema in the population [1,30].

Conversely, the effect of gas density on alveolar gas exchange which should affect all subjects at high altitude is more likely. This hypothesis can be documented on a theoretical and experimental basis. Diffusion in the gas phase depends on many variables that can have opposite effects. Among these, two are prominent: (1) the coefficient of diffusivity (D) of a given gas in a mixture, which increases with decreasing barometric pressure [26] (taken alone, a higher D increases the transfer of gases), and (2) the length of the pathway from the alveolar membrane to the transition point, the latter being set between convection and diffusion in the gas phase. When this length increases with lower gas density, a decrease in the transfer of gases in the airways is apparent [31,32]. At sea level, a 20–30% reduction in the transfer of CO has been measured in subjects breathing a gas mixture of 80% helium/20% oxygen [33], i.e., gas mixture with a high D value and a 33% relative density similar to that of air at 8200 m above sea level. The inverse relationship between alveolar gas exchange and the molecular diffusivity of gas in lung airways has also been documented on a theoretical basis by Nixon and Pack [34]. The decrease in TL_{NO} that we observed in lowlanders at high altitude was 15% of the value at sea level. This decrease is compatible with the fact that the density of air was only reduced to 63% of its value at sea level.

Effect of hypoxia on lung capillary volume

In highlanders, mean TL_{NO} and mean Dm_{CO}/VA ratios were greater than in lowlanders. However, the differences were less pronounced than those in Vc and Vc/VA. These results are consistent with an increase in lung surface area associated with the recruitment of capillaries and a thickening of the capillary blood sheet. In highlanders, Vc was increased by a mean of 91% from sea level reference values. This increase may in part be attributable to lung

hypertrophy that is observed in some highlanders. However, the ratios Vc/VA were above the reference values in all but two highlanders. Recruitment and/or distension of capillaries may be evidenced by this data.

In recruitment hypothesis, both Vc and Dm_{CO} should have been increased by the same proportions. However, Vc increased more (91%) than Dm_{CO} (19%), consistent with the hypothesis of an associated capillary widening due either to an increase in capillary pressure (distension) or to a change in structure. This adaptation was reported on rats, by Howell et al. [4] who demonstrated, using stereological techniques combined with microscopy, that hypoxia led to a greater change in vascular dimensions than could be expected on the basis of distension alone. Another strong argument in favour of recruitment and widening of capillaries comes from a previous study in which the alveolar-capillary structure was assumed to be a rectangular box that exchanged gases through one of its main surfaces. Under this model, the thickness of the box, K, simulates the thickness of the blood sheet while the thickness of the box wall, μ, simulates the membrane thickness. Using this model, the TL_{NO}/TL_{CO} ratio was shown to be inversely related to the product, γ, of the thicknesses K and μ [13]. The relationship is $1/\gamma = ((TL_{NO}/TL_{CO}) - 2) \times B$, where B is a coefficient that has θ_{co} as its only variable. Therefore, the 10% difference in the TL_{NO}/TL_{CO} ratio observed between highlanders (3.6) and lowlanders (3.8) at high altitude can be attributed to a 19% increase in γ. Even if this increase was fully attributed to the blood sheet thickness, it would not explain the +49% difference in Vc/VA between highlanders and lowlanders. We conclude that evidence exists for an associated recruitment of capillaries.

Distension may also be due to the mild increase in pulmonary arterial pressure that combines several mechanisms such as hypoxic arterial pulmonary constriction, muscular arterial hypertrophy, and polycythemia. The main effect of arterial vasoconstriction or muscular arterial hypertrophy is an upstream increase in pressure that should not be followed by a change in downstream pressure. Nevertheless, Wagner et al. [35] found that hypoxic vasoconstriction induced the recruitment of capillaries in dogs due to a better distribution of perfusion associated with increased pulmonary arterial pressure. In the present study, Vc was unchanged in lowlanders despite having a Ppa that was as elevated as in the highlanders. Hypoxia-induced polycythemia may also have consequences. Polycythemia can increase blood viscosity, which in turn induces elevated mean capillary pressure and may conceivably lead to an increase in the capillary blood sheet thickness. *Ex vivo* experiments on the left lower pulmonary lobes of dogs have also suggested that for a given blood flow, an increase in hematocrit might induce the recruitment of capillaries by increasing the perfusion pressure [36]. This phenomenon may contribute to the increase in pulmonary capillary blood volume observed in highlanders.

Pulmonary haemodynamic and capillary lung volume and gas exchange

The increase in pulmonary capillary blood volume may lead to consequences in terms of pulmonary haemodynamics and gas exchange. For a given cardiac blood flow, an increase in Vc increases the blood transit time in the lung, allowing more time for oxygen to equilibrate with the alveolar gas. This increase may be important since altitude hypoxia is known to induce an oxygen diffusion limitation. In addition, an increase in the number and/or the diameter of the pulmonary capillaries might decrease the resistance to blood flow and thereby limit the contribution of capillary resistance to increased pulmonary artery pressure.

The echocardiographic measurements confirmed that exposure to high altitude is associated with moderate pulmonary hypertension and does not vary between native highlanders and recently acclimatised lowlanders [1]. There was a slightly higher left atrial pressure in native highlanders as estimated by Ppao when compared to the acclimatised lowlanders. The increase in the estimated left atrial pressure was an average of 2 mmHg, which is at the limit of the error of measurement, and would not be expected to significantly affect Vc. This higher left atrial pressure may be due to a different volume status and may not be physiologically relevant since the values were no different from those seen at sea level. In an *in vitro* study, the increase in capillary width resulting from a change in venous pressure was estimated to be 2.3% per mmHg [37], which suggests a change in volume of the same order of magnitude, assuming that the capillary bed behaves like a sheet. Therefore with the assumption of a 2 mmHg increase in Ppao, the increase in Vc would be 4.6%; i.e., much less than that observed in highlanders.

Limitation of the study

The two groups of subjects studied here were not perfectly matched as the highlander group was smaller and had a higher BMI than the lowlander group. As highlanders were recruited in a short time either by advertisement in the local newspaper or among the staff of Oruro hospital, it was difficult to obtain an ideal group for comparison with the lowlander group. In our study, mean BMI of the highlander group was 26.2 kg m^2. In a general population which included a majority of subjects not overweight (BMI < 25 kg m^2) and several obese subjects (BMI > 30 kg m^2), Bottai et al. [38] have observed that the changes in TL_{CO} associated with change in BMI were not large enough to be statistically significant. In our highlander group, eight subjects had normal weight, six were overweight and four were obese, which also seems to be representative of a general population. Therefore, if any, the effect of BMI should be small.

Age and height are two other variables known to affect pulmonary diffusion variables [22,39]. Since the highlander group was older (not significantly) and smaller than the lowlander group, they should have had lower pulmonary diffusion values. This means that the marked difference highlighted between the two groups has been under estimated. However, as the calculated predicted values of pulmonary diffusion took into account the effects of gender, height and age [22], the data expressed as percentage predicted values could not have been affected by these variables.

Conclusion

In conclusion, high altitude chronic hypoxia induces an increase in pulmonary capillary blood volume that is disproportionate to that of the membrane surface. This adaptation may benefit pulmonary haemodynamics and gas exchange by decreasing the resistance to blood flow and allowing inhaled oxygen more time to equilibrate with alveolar gas. Distension and recruitment of capillaries are probably both involved in increasing pulmonary capillary blood volume. As hypoxia-induced angiogenesis in the mature pulmonary circulation has been demonstrated by experimental data on rats, the hypothesis of a change in vascular structure can be envisaged to explain the present results in humans. Additional studies using biological markers are needed to confirm the existence of an angiogenic process.

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Annex

Method

The kinetics of CO fixation in respect of haemoglobin were measured in a separate study to assess the conductance of CO with haemoglobin in people from Andean origin.

Four blood samples were studied. They came from subjects born and raised at high altitudes in Bolivia but who had lived in Belgium for several months (three females, one male, 18–42 years of age). One of these individuals was of Aymara descent while the other three were of mixed origins.

Flash. photolysis

Haemoglobin samples of 100 μM were equilibrated on a heme basis under CO in optical cuvettes of 1 mm optical path length. Light transmission at 436 nm was monitored before and after photodissociation using a 10 ns YAG laser pulse at 532 nm (Quantel, France). The photolysis light path was at a right angle to the detection beam, provided by the (Xenon lamp) light source and by the excitation monochrometer of an SLM 8000 fluorometer. The light transmission rate was detected by a photo-multiplier and recorded on a Lecroy (Waverunner LT342L) oscilloscope. A large diameter (6 mm) fibre optic light guide was used to transmit the photolysis beam to the sample cell; this resulted in some loss of intensity, but ensured a more uniform beam. Measurements were made at different laser intensities, with an average of at least 10 kinetic curves at each level [40].

Appendix Results

The recombination kinetics of CO after photodissociation are shown in Fig. 2. For all samples, the traces were biphasic as expected for tetrameric Hb. At low CO photodissociation levels, the CO recombination kinetics of Hb A approach a single fast phase because under these conditions the majority of the photodissociated tetramers are tri-liganded and remain in the R state. At high photodissociation levels, a significant fraction of deoxy and single liganded tetramers are photo-produced. These species switch to the slowly rebinding T-state Hb. One can thus detect a shift in the intrinsic R or T states by a change in rate, and a shift in the allosteric equilibrium by a change in the fraction of rapid to slow phases.

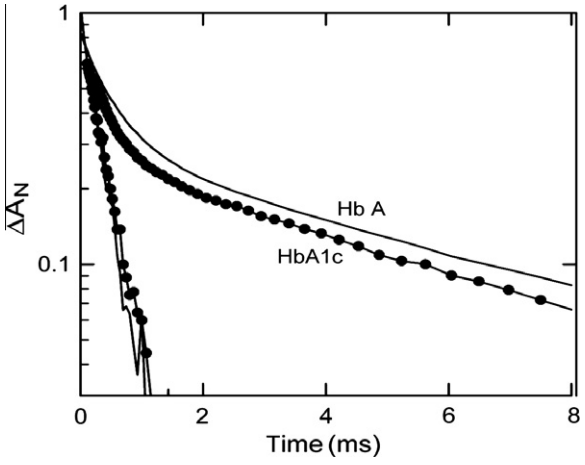

Fig. 2. Recombination kinetics after the photodissociation of CO, for a Bolivian sample of haemoglobin (no different from the general population) and for a sample enhanced in its glycated HbA1c form (about 15%, with data points). Both curves show biphasic kinetics that is characteristic of Hb tetramers. Two levels of photodissociation are shown; at low levels, rapid Hb conformation was observed (left). Curves are normalised to better show the relative amplitudes of the rapid and slow phase.

The percentage of HbA1c in the four samples was not different from that of the general population, about 5%. The kinetics for all samples displayed a form that was typical of Hb tetramers. No new rates that were much faster or slower were observed. If intermediate rates had been present, they would have been more difficult to separate from the usual R and T state phases.

Appendix Discussion

The kinetics of CO recombination are sensitive to changes in the intrinsic R and T states as well as the dynamic transition between these states. The basic rates differ by about a factor of 30 for the pure R and T states. The amplitudes of the relative fractions are well-determined and allow for the detection of shifts in the amount rates of the pure states. No new rates were observed that were beyond the usual range of the R and T states. As a consequence, the CO conductance of haemoglobin was not found to be any different from the general population. The samples came from Bolivians raised at high altitude but living at sea level. Therefore changes may have occurred because of differences in oxygenation or lifestyle. Some forms of haemoglobin may differ from standard HbA in their reactivity with CO. Among these are HbA1c (Fig. 2) and methaemoglobin (MetHb). No difference in HbA1c concentration in non-diabetic populations has been described in high altitude populations, but the concentration of MetHb is reportedly increased in people who live at high altitudes [41]. In polycythemic patients the percentage of MetHb has been reported to be about 4% at high altitudes, returning to normal after a descent to sea level. This form of haemoglobin cannot bind to CO, and therefore the effective haemoglobin concentration at high altitudes may be slightly overestimated.

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